Chromosome numbers of various *Disa* species and their interspecific and complex hybrids

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Polyploid features in some derivatives of *Disa* hybrids prompted an investigation of their chromosome numbers and those of their hybrid parents, the parental species, and a few close relatives. *Disa uniflora* Berg. has $2n = 36$; *D. cardinalis* Linder, *D. racemosa* L.f., *D. tripetaloides* (L.f.) N.E. Br. ssp. *aurata* and *D. venosa* Sw. have $2n = 38$, whereas *D. sagittalis* (L.f.) Sw. and *D. uncinata* H. Bolus have $2n = 40$. *D. tripetaloides* ssp. *tripetaloides* from Joubertsgat is a natural triploid with $2n = 57$. Two of the hybrid derivatives, viz. *Disa* Betty's Bay 'Helderberg' and *Disa* Diores 'Colette' are near tetraploids with $2n = 68$, whereas three others, viz. *Disa* Foam 'Jeanette', *Disa* Helmut Meyer 'Pat' and *Disa* Unimeyer 'Apricot' are near triploids with $2n = 48$, 54 and 52 respectively. The hybrids and especially their robust polyploid derivatives are most attractive and are ideal for the cut-flower industry.

Die poliploïede kenmerke van sommige *Disa*-hibriede se nakomelinge het aanleiding gegee tot 'n sitogenetiese studie van hierdie plante, hul hibriedouers, die spesies wat in die kruisings gebruik is, en 'n paar ander *Disa*-soorte. *Disa uniflora* Berg. het $2n = 36$; *D. cardinalis* Linder, *D. racemosa* L.f., *D. tripetaloides* (L.f.) N.E. Br. ssp. *aurata* en *D. venosa* Sw. het $2n = 38$, terwyl *D. sagittalis* (L.f.) Sw. en *D. uncinata* H. Bolus se chromosoomgetal $2n = 40$ is. *D. tripetaloides* ssp. *tripetaloides* van Joubertsgat is 'n natuurlike triploïed met $2n = 57$. Twee van die hibriedderivate, nl. *Disa* Betty's Bay 'Helderberg' en *Disa* Diores 'Collette' is bykans tetraploïed met $2n = 68$, terwyl drie ander, nl. *Disa* Foam 'Jeanette', *Disa* Helmut Meyer 'Pat' en *Disa* Unimeyer 'Apricot' bykans triploïed is met onderskeidelik $2n = 48$, 54 en 52. Die hibriede en veral hul poliploïede derivate is besonder aantreklik en behoort 'n groot aanwins vir die snyblombedryf te wees.

Keywords: Hybrid derivatives, karyotypes, orchids, polyploids

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Introduction

The genus *Disa* Bergius (Orchidaceae) consists of approximately 124 species, 94 of which are endemic to South Africa (Linder pers. comm.; Linder 1981; Stewart *et al.* 1982). Several of these species were grown and hybridized in England during the previous century. The first hybrid, *D.* Veitchii, from the cross *D. racemosa* L.f. × *D. uniflora* Berg., was registered in 1891 by Veitch in the U.K. Another 11 *Disa* hybrids were registered between 1891 and 1922, but the great potential of the *Disa* hybrids for the cut-flower industry was ignored for more than half a century. In 1962 K.C. Johnson remade *D.* Veitchii in Somerset West (Johnson 1969). A large clone of this hybrid adorned the nursery of H. Meyer in Stellenbosch where it caught the eye of orchid growers. Since 1981 no less than 44 new hybrids have been registered.

Vogelpoel (1985, 1986, 1987) and Cywes *et al.* (1987) reviewed the older *Disa* hybrids including *D.* Kewensis [= *D. uniflora* × *D. tripetaloides* (L.f.) N.E. Br.] and described the more spectacular recent hybrids such as *D.* Veitchii 'Golden Petals' (that resulted from the reciprocal of the cross which gave rise to Veitch's and Johnson's *D.* Veitchii), an F_2 selection from this hybrid that was named *D.* Veitchii 'Ted Schelpe', *D.* Diores (= *D. uniflora* × *D.* Veitchii), *D.* Watsonii (= *D. uniflora* × *D.* Kewensis), *D.* Betty's Bay (= *D.* Diores × *D. uniflora*), *D.* Kirstenbosch Pride (= *D. cardinalis* Linder × *D. uniflora*), *D.* Helmut Meyer (= *D.* Kirstenbosch Pride × *D. uniflora*), *D.* Unimeyer (= *D. uniflora* × *D.* Helmut Meyer), *D.* Kewbett (= *D.* Kewensis × *D.* Betty's Bay), *D.* Unikewbett (= *D. uniflora* × *D.* Kewbett), *D.* Foam (= *D.* Betty's Bay × *D. uniflora*), *D.* Johanna Augustyn (= *D.* Watsonii × *D.* Diores), as well as various mutant variants (see also Vogelpoel *et al.* 1985).

Disas have the advantage over many tropical and subtropical orchids that their seed pods require only 6 weeks instead of 9 months to mature, and they bloom 2 to 3 years after sowing instead of 4 to 5 years. The gene pool constituted by this large and variable genus will enable the production of much genetic and phenotypic variation. Theoretically it should be possible to produce floriferous hybrids with the usual flower colours such as white, pink, red, crimson, orange, yellow, blue, purple as well as black, the pastel shades and harlequins. Three to four closely related genera like *Brownleea* Lindley, *Monadenia* Lindley, *Herschelianthe* Rauschert and *Schizodium* Lindley may prove to be inter-fertile with some *Disa* species, thus extending the gene pool. Helmut Meyer pioneered propagating methods that enabled the ready cultivation of disas.

In orchid hybridization one of the objectives is to

produce triploids and tetraploids, as the features and characteristics of their flowers are more desirable than those of the diploids, both as far as the commercial flowers as well as the show potential of the flowers are concerned.

During the advanced hybridization programme with disas by one of us (S. Cywes) it was observed that some of the segregating progeny had the robust and thicker leaves as well as other characteristics associated with polyploidy. These plants subsequently produced superior flowers. At the same time a higher incidence of infertility was encountered in some of these attractive selections.

Triploids usually have a low fertility. To avoid futile attempts in hybridization with triploid plants, it would be useful to identify them as such by chromosome counts. It will also be very advantageous to know which interspecific hybrids have the amphiploid chromosome number, since they are likely to be fertile and can be selfed and intercrossed to produce a variable range of tetraploids, or back-crossed with their parents to produce triploids, which although infertile, may be of great importance to the flower industry because their flowers have both substance and usually last longer.

The objective of this study was to determine the chromosome numbers of the *Disa* species cultivated at the Kirstenbosch National Botanic Gardens, and of the hybrids that were made available by various growers.

The only references to *Disa* chromosome numbers with which the numbers obtained in the present study could be compared, are those of Hedberg (1977) and of Wimber (1987). Hedberg found a count of $2n = 36$ for *D. stairsii* and Wimber observed $2n = 38$ in *D. tripetaloides* and $2n = 36$ in *D. uniflora*. The four hybrids that he investigated were: *D.* Kewensis (= *D. uniflora* × *D. tripetaloides*), $2n = 37$; *D.* Watsonii (= *D.* Kewensis × *D. uniflora*), $2n = 37$; *D.* Diores (= *D.* Veitchii × *D. uniflora*), $2n = 36, 37, 38, 39$; and *D.* Johanna Augustyn (= *D.* Watsonii × *D.* Diores), $2n = 57$. The latter hybrid is a spontaneous triploid.

Materials and Methods

Mr J. Winter, curator of the Kirstenbosch National Botanic Gardens, kindly provided plants of the *Disa* species listed in Table 1 that were growing in sand-filled pots in their nursery. Root-tips were collected from these species and the following cultivars and hybrids that grew in the greenhouses of H. Meyer* and one of us (S. Cywes):

D. uniflora 'Orange Sensation'
D. uniflora 'W.P. Rugby'
D. uniflora, yellow variant
D. Betty's Bay 'Helderberg' = *D.* Diores × *D. uniflora*, F_2 selection
D. Diores 'Collette' = *D. uniflora* × *D.* Veitchii
D. Foam 'Jeanette' = *D.* Betty's Bay × *D. uniflora*
D. Helmut Meyer 'Pat' = *D.* Kirstenbosch Pride × *D. uniflora*
D. Kewensis = *D. tripetaloides* × *D. uniflora*
**D.* Kewbett = *D.* Kewensis × *D.* Betty's Bay
**D.* Kirstenbosch Pride = *D. cardinalis* × *D. uniflora*
D. Unikewbett = *D. uniflora* × *D.* Kewbett
D. Unimeyer = *D. uniflora* × *D.* Helmut Meyer
**D.* Veitchii = *D. uniflora* × *D. racemosa*
D. Veitchii 'Ted Schelpe' = *D. uniflora* × *D. racemosa*, F_2 selection
D. Watsonii 'Boland' = *D. uniflora* × *D.* Kewensis
D. Watsonii 'Kolman' = *D. uniflora* × *D.* Kewensis

Variations of the pretreatment, fixation and staining methods described by Darlington & La Cour (1960) for plant material were tried and compared with the method of Wimber (1987). The best results were obtained with the following schedule:

Between 08h00 and 11h00, actively growing white roots were cut 10 mm from the meristematic tip. After rinsing them to remove all the sand particles, they were placed in vials containing 5 ml of a freshly made α-bromonaphthalene solution (1 drop shaken in 250 ml double distilled water) to prevent spindle formation and arrest the divisions at metaphase. The vials were kept in the dark at room temperature (21°C) for 4 to 4.5 h. The roots were then fixed in a freshly made fixative consisting of 4 parts methanol and 1 part propionic acid (stirring every few minutes for the first 15 min) for 24 h (the roots can be stored in the fixative at 4°C, but 70% ethanol is preferable). After fixation the roots were rinsed in distilled water for 30 min and then hydrolysed in 1N HCl at 60°C for 6.5 minutes. They were stained in 1 to 2 ml cold Feulgen stain [prepared according to Darlington & La Cour (1960), using BDH pararosanalin instead of the prescribed basic fuchsin] for 2 h. The roots were rinsed twice in distilled water for 5 min and placed in a pH 4.5 sodium acetate buffer solution for 5 min. The buffer was then replaced by a pectinase solution consisting of 1 g Spark L pc (by Seravac) and 0.2 g peptone (Difco) in 20 ml of the above buffer solution. The roots were kept in this solution for 30 min to 1 h at 37°C. The meristematic tip of each root was cut off in a small drop of 1% aceto-carmine (made according to Darlington & La Cour 1960), the cells separated by gentle tapping with a hardwood needle holder, and the larger segments removed. The drop was covered with an albuminized cover-slip, and the preparation squashed under folded filter paper. Gentle heating over a spirit flame and further squashing were required to flatten the chromosome spreads to the same focus plane. Permanent preparations were made by inverting the slides in a dish with 45% acetic acid till the cover-slips floated off. The cover-slips were passed through a solution consisting of equal parts 45% acetic acid and tertiary butyl alcohol, followed by two changes of pure tertiary butyl alcohol for 2 min in each, and mounted in Canada Balsam on clean slides. All microscopic observations were made with a Standard Lab 16 Zeiss microscope equipped with a 100× Neofluar 1.3 NA objective and Kpl-W 12.5×/18 Br eyepieces. The photomicrographs were taken with a Zeiss photomicroscope on Kodak Technical Pan TP 135-36 film and developed in HC-110 developer (dilution D) for 6 min at 20°C.

Table 1 Chromosome numbers of *Disa* species and hybrids

	Locality/source	NBG code no.	2*n* Present study	2*n* [1]Hedberg (1977) [2]Wimber (1987)
(a) *Disa* species				
1. *D. cardinalis* Linder	Riversdale	192/79	38	
2. *D. racemosa* L.f.	Botrivier	60/80	38	
3. *D. sagittalis* (L.f.) Sw.	Port Edward	341/83	40	
4. *D. tripetaloides* (L.f.) N.E. Br.				38[2]
ssp. *tripetaloides*	Jan Joubertsgat	705/83	57	
5. ssp. *aurata* (H. Bolus) Linder	Swellendam	1202/84	38	
6. *D. uncinata* H. Bolus	Franschhoek	447/82	40	
7. *D. uniflora* Berg.	Beacon Ridge	54/80	36	
D. uniflora Berg.				36[2]
8. *D. uniflora* Berg.	Betty's Bay	61/80	36	
9. *D. uniflora* Berg.	Fonteintjiesberg	56/80	36	
10. *D. uniflora* Berg.	Groot Winterhoek Mts.	708/83	36	
11. *D. uniflora* Berg.	Kirstenbosch Table Mountain	57/80	36	
12. *D. uniflora* yellow variant	Cywes	*	36	
13. *D. uniflora* 'Orange Sensation'	Cywes	*	36	
14. *D. uniflora* 'W.P. Rugby'	Cywes	*	36	
15. *D. venosa* Sw.	Seweweekspoort	675/84	38	
16. *D. stairsii*				36[1]
(b) *Disa uniflora* hybrids				
17. *D.* Betty's Bay 'Helderberg'	Cywes	*	68	
18. *D.* Diores				36–39[2]
19. *D.* Diores 'Colette'	Cywes	*	68	
20. *D.* Foam 'Jeanette'	Cywes	*	48	
21. *D.* Helmut Meyer 'Pat'	Cywes	*	54	
22. *D.* Kewensis	Meyer	*	37	
D. Kewensis				37[2]
23. *D.* Kirstenbosch Pride	Meyer	*	38	
24. *D.* Unikewbett	Cywes	*	36	
25. *D.* Unimeyer 'Apricot'	Cywes	*	52	
26. *D.* Veitchii	Meyer	*	37	
27. *D.* Veitchii 'Ted Schelpe'	Cywes	*	36	
28. *D.* Watsonii 'Boland'	Cywes	*	36	
D. Watsonii				37[2]
29. *D.* Watsonii 'Kolman'	Cywes	*	36	
30. *D.* Johanna Augustyn				57[2]

*Photographs are available in the library of the Disa Orchid Society

Results and Discussion

The chromosome counts of the above material are given in Table 1 and illustrated in Figure 1A–P. All seven species investigated have chromosome numbers characteristic for the Orchidaceae, viz., $2n = 36$, 38 or 40 (Darlington & Wylie, (1956). The chromosome numbers found in *D. tripetaloides* and *D. uniflora* agree with those of Wimber (1987) for these species. However, the white-pink sub-species of *D. tripetaloides* from Joubertsgat proved to be a natural triploid with $2n = 57$ (Figure 1D). The interphase nuclei of all the *Disa* species contain many chromocenters (Figure 1L) indicating that many of the chromosomes have large segments of heterochromatin. This explains the stickiness of the metaphase chromosomes which made accurate counts of the small chromosomes very difficult. This was especially true when a cold-water pretreatment was used instead of the α-bromonophthalene solution (Figure 1O & P). Several metaphase spreads had to be counted to obtain a reasonably reliable somatic chromosome number. The 36 chromosomes of *D. uniflora* consist of three pairs of larger chromosomes (including a satellited pair), three pairs of medium-sized chromosomes and 12 pairs of small chromosomes (see karyotype in Figure 1M). Most of the chromosomes are telocentric or subtelocentric, but a few of the larger chromosomes are acrocentric.

The interspecific hybrids, as expected, have a chromosome number equal to the sum of the haploid number of

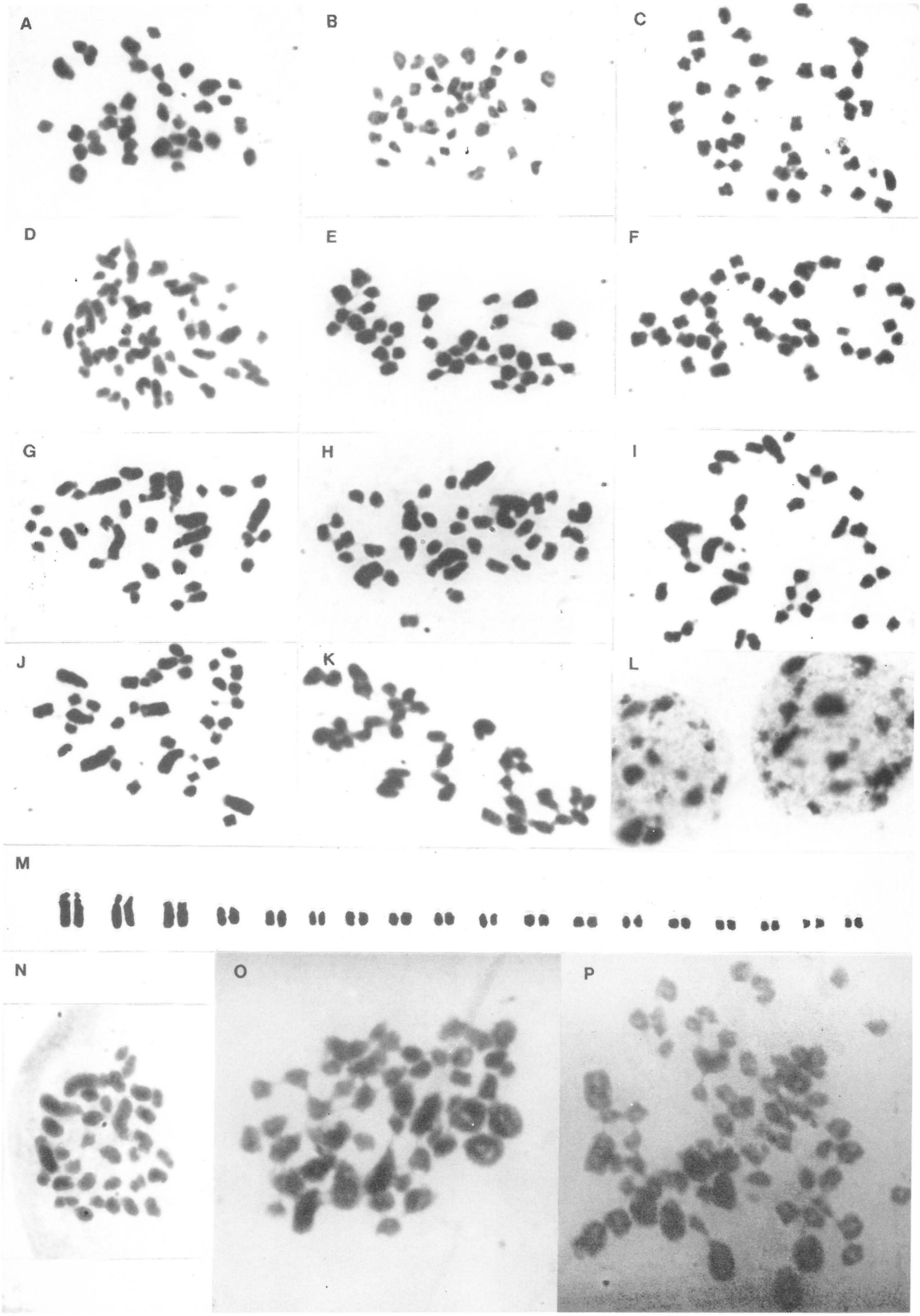

Figure 1A–K Metaphase chromosome spreads in squashed root-tip cells of *Disa* species. (A) *Disa cardinalis* (Riversdale) with $2n = 38$; (B) *D. racemosa* (Botrivier) with $2n = 38$; (C) *D. sagittalis* (Port Edward) with $2n = 40$; (D) *D. tripetaloides* spp. *tripetaloides*, white-pink (Jan Joubertsgat) with $2n = 3x = 57$; (E) *D. tripetaloides* ssp. *aurata* (Swellendam) with $2n = 38$; (F) *D. uncinata* (Franschhoek) with $2n = 40$; (G) *D. uniflora* (Beacon Ridge) with $2n = 36$; (H) *D. uniflora* (Kirstenbosch) with $2n = 36$; (I) *D. uniflora* (Groot Winterhoek) with $2n = 36$; (J) *D. uniflora* (Fonteintjiesberg) with $2n = 36$; (K) *D. venosa* (Seweweekspoort) with $2n = 38$. **L.** Chromocentres in somatic cell nuclei of *D. uniflora*. **M.** *D. uniflora* (Fonteintjiesberg) karyotype showing 18 chromosome pairs. **N–P.** Metaphase chromosome spreads in squashed root-tip cells of *Disa* hybrids. (N) *D.* Kewensis with $2n = 37$; (O) *D.* Unimeyer 'Apricot' with $2n =$ near $3x = 52$; (P) *D.* Betty's Bay 'Helderberg' with $2n =$ near $4x = 68$.

Figure 2 *D.* Betty's Bay 'Helderberg' AM/DOSA. Near-tetraploid, $2n = 68$.

Figure 3 *D.* Diores 'Colette' AM/DOSA. Near-tetraploid, $2n = 68$.

Figure 4 *D.* Foam 'Jeanette' AM/DOSA. Near-triploid, $2n = 48$.

Figure 5 *D.* Unimeyer 'Apricot' AM/DOSA. Near-triploid, $2n = 52$.

Figure 6 *D.* Helmut Meyer 'Pat' FCC/DOSA. Near-triploid, $2n = 54$.

the two parents, e.g., *D.* Kewensis (= *D. tripetaloides*, $2n = 38 \times$ *D. uniflora*, $2n = 36$) have $2n = 37$, and *D.* Veitchii (= *D. uniflora*, $2n = 36 \times$ *D. racemosa*, $2n = 38$) have $2n = 37$. The more complex hybrids have chromosome numbers ranging from 36 to 38. Several of the hybrid derivatives are spontaneous 'polyploids' (near triploids and tetraploids) that probably arose from 'unreduced' gametes as a consequence of restitution nucleus formation during meiotic failure in the hybrid parents. Therefore *D.* Betty's Bay 'Helderberg' and *D.* Diores 'Collette' (Figures 2 & 3) are near-tetraploids, whereas *D.* Foam 'Jeanette', *D.* Unimeyer 'Apricot' (Figures 4 & 5), *D.* Helmut Meyer 'Pat' (Figure 6), and *D.* Johanna Augustyn are triploids or near-triploids (Table 1 and Figures 1O & P). This explains the low fertility of the latter four hybrid derivatives.

These polyploids have much larger and more attractive flowers than the diploids, and have a great potential as cut flowers. Indeed several have won awards on various flower shows and *D.* Helmut Meyer 'Pat' (Figure 6), a triploid, was the first disa ever to receive a First Class Certificate (FCC/DOSA) .

This investigation will be continued with other *Disa* species and hybrids in post-graduate research projects.

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